PHOTOCHEMICAL EFFECT OF LASER-EXCITED HEMATOPORPHYRIN DYE
ON BLAST LYMPHOCYTES

Masayoshi SHIMAMOTO, Susumu KIKUCHI, Shunichi SATO, Humio INABA,
Susumu SATOMI*, Yoshio TAGUCHI* and Morio KASAI*

Research Institute of Electrical Communication,
Tohoku University, Katahira 2-1-1, Sendai 980, Japan

* The 2nd Department of Surgery, School of Medicine,
Tohoku University, Seiryō-cho 1-1, Sendai 980, Japan

It is known that organic dyes such as hematoporphyrin(Hp) or its derivative(HpD) exhibit cytocidal activity on normal and tumor cells through the photoradiation of visible light[1]. This property along with the accumulation in tissues, with a certain degree of specificity, leads to the attractive treatment of tumors, called photoradiation therapy(PRT). The activation process involves the intermediacy of the cytotoxic agent of the singlet molecular oxygen(1O_2) generated by the intersystem crossing from excited triplet dyes, which is known as photochemical or photodynamic action[2]. As a further extension of the PRT concept motivated by finding new facts and applications at basic and clinical levels, this paper reports, for the first time, the experimental study of photochemical effect on blast lymphocytes uptaken Hp species. Blast lymphocytes are recognized clinically, for instance, to be associated with the rejection in organ transplantations, such as renal one.

The lymphocytes used in this experiment were derived from healthy people. These normal cells were stimulated in a medium containing concanavalin A (Con A) at 37°C for 3 days to obtain blast lymphocytes. Then both the normal and blast lymphocytes were incubated in PBS containing hematoporphyrin dihydrochloride (Hp.2HCl), with the concentration of 1×10^{-4} , 5×10^{-5} , 2×10^{-5} , 1×10^{-5} mol/l, at 37°C for 1 hour. For the excitation, all emission lines of an Ar laser was employed. Thus the survival rates of both the sample cells were measured after the irradiation. Also the intensity distribution of dye fluorescence across individual cells as well as their fluorescence images were examined and compared using a very high sensitive laser-excited fluorescence microscope system which was newly developed in our laboratory[3,4].

Figure 1 (a) and (b) show TV-monitor displayed pictures of a conventional microscope image of a blast lymphocyte containing Hp.2HCl and its fluorescence image detected by an ultra-high sensitive SIT camera utilized in our system, respectively. Moreover, a three dimensional graphic display of the fluorescence intensity distribution across the cell is depicted in Fig.1(c). It is found that the fluorescence intensity is stronger in cytoplasm than the nuclear portion in the blast lymphocyte. Also the comparison with the normal lymphocyte reveals that its total fluorescence intensity is about four times weaker than that of the blast one.

Figure 2 (a) and (b) illustrate the measured survival rate of blast and normal lymphocytes uptaken different Hp.2HCl concentrations

as a function of irradiation time of 10 mW/cm^2 laser power density, respectively. We can see that the survival rate shows generally a linear decrease with the irradiation time, and this effect is more appreciable for higher dye concentration, although there exists certainly larger survival rate for the normal lymphocyte, compared with the blast one, even for the same dye concentration and irradiation time. In Fig.3 (a) and (b), the inactivation coefficients are plotted against the dye concentration for both lymphocytes. Here, we defined the gradient of survival rate as the inactivation coefficient. It is found for the first time that this coefficient for the blast cell is larger and shows steeper change against the dye concentration than that for the normal cell.

Though this novel finding of laser-induced photochemical effect for the blast lymphocyte needs to be investigated fully, the phenomenon is likely to be associated with the fact that the normal lymphocyte generally uptakes less Hp dye in comparison with the blast one. This photochemical effect selective to the blast cell based on the uptake of specific dyes seems to be promising for possible applications to immunological cell phototherapy, as a fascinating extension of cancer PRT, in the future.

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Fig.1 Microscope picture (a) and fluorescence image (b) displayed on TV monitor employing a SIT camera. (c) 3-D graphic display of fluorescence intensity distribution across a blast lymphocyte containing Hp.2HCl.

Fig.2 Measured survival rates of blast lymphocytes (a) and normal ones (b) vs. Ar laser irradiation time.

Fig.3 Inactivation coefficients of blast lymphocytes (a) and normal ones (b) vs. Hp.2HCl concentration after Ar laser irradiation.